Translation

PCT Application
PCT/JP2003/005464

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PH-1796-PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/JP03/05464	International filing date (*day/month/year*) 28 April 2003 (28.04.03)	Priority date (*day/month/year*) 26 April 2002 (26.04.02)
International Patent Classification (IPC) or national classification and IPC C12N 15/09, 1/19, 9/04, 9/10, 9/50 // C12R 1:645		
Applicant	KIRIN BEER KABUSHIKI KAISHA	

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

CORRECTED VERSION

Date of submission of the demand 28 April 2003 (28.04.03)	Date of completion of this report 04 November 2003 (04.11.2003)
Name and mailing address of the IPEA/JP	Authorized officer
Facsimile No.	Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/JP03/05464

I. Basis of the report

1. With regard to the elements of the international application:*

☒ the international application as originally filed

☐ the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the claims:
pages _____, as originally filed
pages _____, as amended (together with any statement under Article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the drawings:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

ternational application No.
PCT/JP03/05464

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

SEE SUPPLEMENTAL SHEET

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. _____.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: IV.3

The inventions of the present international application can be classified into the following groups:
(1) claims 1-25 and 94-122: inventions pertaining to methods for producing a methylotroph yeast that is capable of producing a mammalian-type sugar chain;
(2) claims 26-30: inventions pertaining to an orotidine-5'-phosphate decarboxylase (URA3) gene;
(3) claims 31-35: inventions pertaining to a phosphoribosyl-amino-imidazole succinocarboxamide synthase (ADE1) gene;
(4) claims 36-40: inventions pertaining to an imidazole-glycerol-phosphate dehydratase (HIS3) gene;
(5) claims 41-45: inventions pertaining to a 3-isopropylmalate dehydrogenase (LEU2) gene;
(6)claims 46-49: inventions pertaining to an α-1,6-mannosyltransferase (OCH1) gene;
(7) claims 50-53: inventions pertaining to a PEP4 gene;
(8) claims 54-57: inventions pertaining to a proteinase B (PRB1) gene;
(9) claims 58-69: inventions pertaining to a YPS1 gene;
(10) claims 70-73: inventions pertaining to a KTR1 gene;
(11) claims 74-77: inventions pertaining to an MNN9 gene;
(12) claims 78-85: inventions pertaining to an alcohol oxidase (AOX) gene; and
(13) claims 86-93: inventions pertaining to a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene.

However, the methods for producing a methylotroph yeast that is capable of producing a mammalian-type sugar chain in group (1), and the inventions related to the orotidine-5'-phosphate decarboxylase (URA3) gene in group

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: IV.3

(2), the phosphoribosyl-amino-imidazole succinocarboxamide synthase (ADE1) gene in group (3), the imidazole-glycerol-phosphate dehydratase (HIS3) gene in group (4), the 3-isopropylmalate dehydrogenase (LEU2) gene in group (5), the α-1,6-mannosyltransferase (OCH1) gene in group (6),the PEP4 gene in group (7), the proteinase B (PRB1) gene in group (8), the YPS1 gene in group (9), the KTR1 gene in group (10), the MNN9 gene in group (11), the alcohol oxidase (AOX) gene in group (12), and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene in group (13) were all well-known prior to the priority date of the present application (refer to "C. Documents Considered to be Relevant" in the international search report); therefore, these features cannot be considered to be special technical features in the light of the prior art.

Consequently, these 13 groups of inventions cannot be considered to be a group of inventions so linked as to form a single general inventive concept.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/JP 03/05464

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	4-5, 8-9, 15, 17-22, 26-122	YES
	Claims	1-3, 6-7, 10-14, 16, 23-25	NO
Inventive step (IS)	Claims		YES
	Claims	1-122	NO
Industrial applicability (IA)	Claims	1-122	YES
	Claims		NO

2. Citations and explanations

Document 1: WO 02/00856 A2 (Flanders Interuniversity Institute for Biotechnology), 03 January 2002, claims, examples, & US 2002/188109 A & EP 1294910 A2

Document 2: WO 02/00879 A2 (Glycofi Inc.), 03 January 2002, claims, table 3, examples, & US 2002/137134 A & EP 1297172 A2

Document 3: Yasuyoshi SAKAI et al., "The Orotidine-5'-Phosphate Decarboxylase Gene (URA3) of a Methylotrophic Yeast, *Candida boidinii*: Nucleotide Sequence and its Expression in *Escherichia coli*," Journal of Fermentation and Bioengineering, 1992, Vol. 73(4), pages 255-260, entire document, especially fig. 2

Document 4: Vina W. YANG et al., "High-Efficiency Transformation of *Pichia stipitis* Based on its URA3 Gene and a Homologous Autonomous Replication Sequence, ARS2," Applied and Environmental Microbiology, 1994, Vol. 60(12), pages 4245-4254, entire document, especially fig. 3

Document 5: Yoshiaki NISHIYA et al., "Primary Structure of ADE1 Gene from *Candida utilis*," Bioscience Biotechnology and Biochemistry,

1994, Vol. 58(1), pages 208-210, entire document, especially fig. 3

Document 6: Inmaculada C. COSANO et al., "Cloning and Sequence Analysis of the *Pichia pastoris* TRP1, IPP1 and HIS3 Genes," Yeast, 1998, Vol. 14, pages 861-867, entire document, especially fig. 4

Document 7: WO 98/14600 A1 (Centro de Ingenieria y Biotecnologia), 09 April 1998, claims, SEQ ID NO: 5-6, & JP 2001-501475 A & EP 956356 A1

Document 8: Yasuyoshi SAKAI et al., "Directed Mutagenesis in an Asporogenous Methylotrophic Yeast: Cloning, Sequencing and One-step Gene Disruption of the 3-Isopropylmalate Dehydrogenase Gene (LEU2) of *Candida boidinii* to Derive Doubly Auxotrophic Marker Strains," Journal of Bacteriology, 1992, Vol. 174(18), pages 5988-5993, entire document, especially fig. 2

Document 9: Ying-Pei ZHANG et al., "LEU2 Gene Homolog in *Kluyveromyces lactis*," Yeast, 1992, Vol. 8, pages 801-804, entire document, especially fig. 1

Document 10: JP 9-3097 A (The Green Cross Corp.), 07 January 1997, claims, SEQ ID NO: 5, fig. 5, (Family: none)

Document 11: WO 00/14259 A1 (Kirin Brewery Co., Ltd.), 16 March 2000, claims, SEQ ID NO: 2-3, & JP 2000-78978 A

Document 12: WO 92/17595 A1 (The Salk Institute Biotechnology/Industrial Associates), 15 October 1992, claims, SEQ ID NO: 1-2, & JP 6-506117 A & EP 578746 A1 & US 5324660 A

Document 13: Anahit V. AZARYAN et al., "Purification and Characterization of a Paires Basic Residue-specific Yeast Aspartic Protease Encoded by the YAP3 Gene," The Journal of Biological Chemistry, 1993, Vol. 268(16), pages 11968-11975, entire document

Document 14: Hiroto KOMANO et al., "Shared Functions in vivo of a Glycosyl-phosphatidylinositol-linked aspartyl protease, Mkc7, and the Proprotein Processing Protease Kex2 in Yeast," Proc. Natl. Acad. Sci. USA, 1995, Vol. 92, pages 10752-10756, entire document, especially fig. 2

Document 15: Ed T. BUURMAN et al., "Molecular Analysis of CaMnt1p, a Mannosyl Transferase Important for Adhesion and Virulence of *Candida albicans*," Proc. Natl. Acad. Sci. USA, 1998, Vol. 95, pages 7670-7675, entire document, especially fig. 1

Document 16: EP 314096 A2 (Zymogenetics, Inc.), 03 May 1989, claims, fig. 4, & JP 2-419 A & US 5135854 A & DE 3887082 A

Document 17: A. M. LEDEBOER et al., "Molecular Cloning and Characterization of a Gene Coding for Methanol Oxidase in *Hansenula polymorpha*," Proc. Natl. Acad. Sci. USA, 1998, Vol. 95, pages 7670-7675, entire document, especially fig. 6

Document 18: EP 173378 A2 (Nnilever PLC), 05 March 1986, claims, fig. 11 and 13, & JP 61-92569 A & US 5240838 A & DE 3583194 A

Document 19: WO 00/78978 A1 (Zymogenetics Inc.), 28 December 2000, claims, SEQ ID NO 1-2, & JP 2003-503030 A & EP 1192263 A1

Document 1 discloses a method for producing proteins that have a mammalian-type sugar chain by introducing vectors that express α-1,2-mannosidase into a methylotroph yeast, and specifically discloses features wherein vectors that express α-1,2-mannosidase are introduced into a *Pichia*-species yeast and the Och1 genes are deactivated. Furthermore, document 1 indicates that AOXI, AOXII, GAP and the like can be selected as the promoter for said vectors, and that ER retention signals are added to the α-1,2-mannosidase genes.

Document 2 discloses a method for producing proteins that have a mammalian-type sugar chain, and specifically discloses the features of producing mutants of *Pichia pastorus* that do not express OCH1 and of transforming the mutants so that they express mannosidase. Document 2 also indicates that the α-1,2-mannosidase is obtained from microscopic organisms such as *Aspergillus saitoi*.

Claims 1-3, 6-7, 10-14, 16 and 23-25 lack novelty in the light of the disclosures of document 1.

Claims 1-3, 10-12 and 23-25 lack novelty in the light of the disclosures of document 2.

In addition, it would be easy for a person skilled in the art to apply these features to *Ogataea minuta*, which is one type of methylotroph yeast, to express α-1,2-mannosidase using a promoter such as AOXI, AOXII or GAP, and to obtain a protein that has a desired N-type sugar chain by deactivating the gene related to the production of the sugar chain and introducing an appropriate heterogeneous gene.

Therefore, it is considered to have been easy for a person skilled in the art to conceive of the inventions that are set forth in claims 1-25 and 94-122 in the light of documents 1-4.

Documents 3-4 disclose the URA3 gene from *Candida boidinii* and the URA3 gene from *Pichia stipitis*.

Document 5 discloses the ADE1 gene from *Candida utilis*.

Documents 6-7 disclose the HIS3 gene from *Pichia pastoris* and the HIS3 gene from *Candida utilis*.

Documents 8-9 disclose the LEU2 gene from *Candida boidinii* and the LEU2 gene from *Kluyveromyces lactis*.

Document 10 discloses α-1,6-mannosidase from a *Pichia*-species yeast and the gene that codes said mannosidase, and indicates that this gene is homologous to the OCH1 gene.

Document 11 discloses proteases A and B from *Candida boidinii* and the genes that code said proteases.

Document 12 discloses protease A from a *Pichia*-species yeast and the gene that codes said protease.

Documents 13-14 disclose the protease YAP3 from yeast and the gene that codes said protease.

Document 15 discloses the KTR1 gene from *Saccharomyces cerevisiae*.

Document 16 discloses the MNN9 gene from *Saccharomyces cerevisiae*.

Documents 17-18 disclose the MOX gene from *Saccharomyces cerevisiae* and *Hansenula polymorpha*.

Document 19 discloses the GAP1 gene from *Pichia methanolica*, and the promoter and terminator therefor.

On the priority date for the present application, it was common practice in this technical field to synthesize a probe or primer from a portion of a known base sequence that codes a useful natural protein in order to clone the same DNA chain from a different natural source (refer to, for example, Pro. N.A.S., Vol. 72, 1975, pages 3961-3965); therefore, it is not considered to be especially difficult for a person skilled in the art to use probes that are synthesized from portions of the base sequences of each of the genes that are disclosed in

documents 3-19 in order to obtain the URA3 gene, ADE1 gene, HIS3 gene, LEU2 gene, OCH1 gene, PEP4 gene, PRB1 gene, YPS1 gene, KTR1 gene, MNN9 gene, AOX gene, GAPDH gene and the promoters and terminators of the AOX gene and GAPDH gene from *Ogataea minuta*.

In addition, it is common practice for a person skilled in the art to create recombined vectors that contain said genes, transform *Ogataea minuta* using said recombinant vectors, and produce proteins by cultivating the obtained transformants.

Therefore, it is considered to have been easy for a person skilled in the art to conceive of the inventions that are set forth in claims 26-93 in the light of documents 3-19.